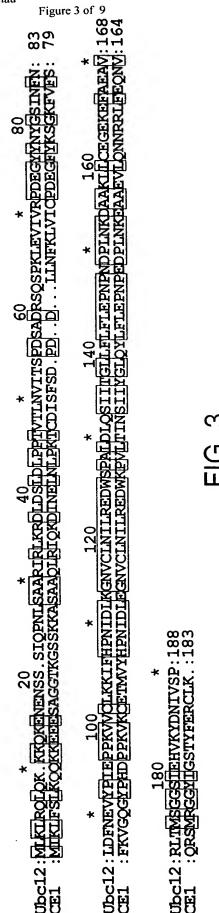Title: HUMAN PROTEINS RESPONSIBLE FOR NEDD8
ACTIVATION AND CONJUGATION
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Attorney Docket No.: MPI97-057P1RCP1CN1M
Applicant: Vinvent Chau

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```
ATGGCTGTTGATGGTGGGTGTGGGGACACTGGAGACTGGGAAGGT    45
  M   A   V   D   G   G   C   G   D   T   G   D   W   E   G
CGCTGGAACCATGTAAAGAAGTTCCTCGAGCGATCTGGACCCTTC    90
  R   W   N   H   V   K   K   F   L   E   R   S   G   P   F
ACACACCCTGATTTCGAACCGAGCACTGAATCTCTCCAGTTCTTG    135
  T   H   P   D   F   E   P   S   T   E   S   L   Q   F   L
TTAGATACATGTAAAGTTCTAGTCATTGGAGCTGGCGGCTTAGGA    180
  L   D   T   C   K   V   L   V   I   G   A   G   G   L   G
TGTGAGCTCCTGAAAAATCTGGCCTTGTCTGGTTTTAGACAGATT    225
  C   E   L   L   K   N   L   A   L   S   G   F   R   Q   I
CATGTTATAGATATGGACACTATAGATGTTTCCAATCTAAATAGG    270
  H   V   I   D   M   D   T   I   D   V   S   N   L   N   R
CAGTTTTTATTTAGGCCTAAAGATATTGGAAGACCTAAGGCTGAA    315
  Q   F   L   F   R   P   K   D   I   G   R   P   K   A   E
GTTGCTGCAGAATTTCTAAATGACAGAGTTCCTAATTGCAATGTA    360
  V   A   A   E   F   L   N   D   R   V   P   N   C   N   V
GTTCCACATTTCAACAAGATTCAAGATTTTAACGACACTTTCTAT    405
  V   P   H   F   N   K   I   Q   D   F   N   D   T   F   Y
CGACAATTTCATATTATTGTATGTGGACTGGACTCTATCATCGCC    450
  R   Q   F   H   I   I   V   C   G   L   D   S   I   I   A
AGAAGATGGATAAATGGCATGCTGATATCTCTTCTAAATTATGAA    495
  R   R   W   I   N   G   M   L   I   S   L   L   N   Y   E
GATGGTGTCTTAGATCCAAGCTCCATTGTCCCTTTGATAGATGGG    540
  D   G   V   L   D   P   S   S   I   V   P   L   I   D   G
GGGACAGAAGGTTTTAAAGGAAATGCCCGGGTGATTCTGCCTGGA    585
  G   T   E   G   F   K   G   N   A   R   V   I   L   P   G
ATGACTGCTTGTATCGAATGCACGCTGGAACTTTATCCACCACAG    630
  M   T   A   C   I   E   C   T   L   E   L   Y   P   P   Q
GTTAATTTTCCCATGTGCACCATTGCATCTATGCCCAGGCTACCA    675
  V   N   F   P   M  (C*)  T   I   A   S   M   P   R   L   P
GAACACTGTATTGAGTATGTAAGGATGTTGCAGTGGCCTAAGGAG    720
  E   H   C   I   E   Y   V   R   M   L   Q   W   P   K   E
CAGCCTTTTGGAGAAGGGGTTCCATTAGATAGAGATGATCCTGAA    765
  Q   P   F   G   E   G   V   P   L   D   G   D   D   P   E
CATATACAATGGATTTTCCAAAAATCCCTAGAGAGCATCACAA    810
  H   I   Q   W   I   F   Q   K   S   L   E   R   A   S   Q
TATAATATTAGGGGTGTTACGTATAGGCTCACTCAAGGGGTAGTA    855
  Y   N   I   R   G   V   T   Y   R   L   T   Q   G   V   V
AAAAGAATCATTCCTGCAGTAGCTTCCACAAATGCAGTCATTGCA    900
  K   R   I   I   P   A   V   A   S   T   N   A   V   I   A
GCTGTGTGTGCCACTGAGGTTTTTAAAATAGCCACAAGTGCATAC    945
  A   V   C   A   T   E   V   F   K   I   A   T   S   A   Y
ATTCCCTTGAATAATTACTTGGTGTTTAATGATGTAGATGGGCTG    990
  I   P   L   N   N   Y   L   C   F   N   D   V   D   G   L
TATACATACACATTTGAAGCAGAAAGAAAGGAAAACTGCCCAGCT    1035
  Y   T   Y   T   F   E   A   E   R   K   E   N   C   P   A
TGTAGCCAGCTTCCTCAAAATATTCAGTTTTCTCCATCAGCTAAA    1080
  C   S   Q   L   P   Q   N   I   Q   F   S   P   S   A   K
CTACAGGAGGTTTTGGATTATCTAACCAATAGTGCTTCTCTGCAA    1125
  L   Q   E   V   L   D   Y   L   T   N   S   A   S   L   Q
ATGAAATCTCCAGCCATCACAGCCACCCTAGAGGGAAAAAATAGA    1170
  M   K   S   P   A   I   T   A   T   L   E   G   K   N   R
ACACTTTACTTACAGTCGGTAACCTCTATTGAAGAACGAACAAGG    1215
  T   L   Y   L   Q   S   V   T   S   I   E   E   R   T   R
CCAAATCTCTCCAAAACATTGAAAGAATTGGGGCTTGTTGATGGA    1260
  P   N   L   S   K   T   L   K   E   L   G   L   V   D   G
CAAGAACTGGCGGTTGCTGATGTCACCACCCCACAGACTGTACTA    1305
  Q   E   L   A   V   A   D   V   T   T   P   Q   T   V   L
TTCAAACTTCATTTTACTTCTTAA    1329
  F   K   L   H   F   T   S   .
```

FIG. 1

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```
+1    M    I    K    L    F    S    L    K    Q    Q    K    K    E    E    E    S    A
1     ATGATCAAGC TGTTCTCGCT GAAGCAGCAG AAGAAGGAGG AGGAGTCGGC
      TACTAGTTCG ACAAGAGCGA CTTCGTCGTC TTCTTCCTCC TCCTCAGCCG
--------------------------------------------------------------------------------
+1    G    G    T    K    G    S    S    K    K    A    S    A    A    Q    L    R
51    GGGCGGCACC AAGGGCAGCA GCAAGAAGGC GTCGGCGGCG CAGCTGCGGA
      CCCGCCGTGG TTCCCGTCGT CGTTCTTCCG CAGCCGCCGC GTCGACGCCT
--------------------------------------------------------------------------------
+1    I    Q    K    D    I    N    E    L    N    L    P    K    T    C    D    I    S
101   TCCAGAAGGA CATAAACGAG CTGAACCTGC CCAAGACGTG TGATATCAGC
      AGGTCTTCCT GTATTTGCTC GACTTGGACG GGTTCTGCAC ACTATAGTCG
--------------------------------------------------------------------------------
+1    F    S    D    P    D    D    L    L    N    F    K    L    V    I    C    P    D
151   TTCTCAGATC CAGACGACCT CCTCAACTTC AAGCTGGTCA TCTGTCCTGA
      AAGAGTCTAG GTCTGCTGGA GGAGTTGAAG TTCGACCAGT AGACAGGACT
--------------------------------------------------------------------------------
+1    E    G    F    Y    K    S    G    K    F    V    F    S    F    K    V    G
201   TGAGGGCTTC TACAAGAGTG GGAAGTTTGT GTTCAGTTTT AAGGTGGGCC
      ACTCCCGAAG ATGTTCTCAC CCTTCAAACA CAAGTCAAAA TTCCACCCGG
--------------------------------------------------------------------------------
+1    Q    G    Y    P    H    D    P    P    K    V    K    C    E    T    M    V    Y
251   AGGGTTACCC GCATGATCCC CCCAAGGTGA AGTGTGAGAC AATGGTCTAT
      TCCCAATGGG CGTACTAGGG GGGTTCCACT TCACACTCTG TTACCAGATA
--------------------------------------------------------------------------------
+1    H    P    N    I    D    L    E    G    N    V   Ⓒ   L    N    I    L    R    E
301   CACCCCAACA TTGACCTCGA GGGCAACGTC TGCCTCAACA TCCTCAGAGA
      GTGGGGTTGT AACTGGAGCT CCCGTTGCAG ACGGAGTTGT AGGAGTCTCT
--------------------------------------------------------------------------------
+1    D    W    K    P    V    L    T    I    N    S    I    I    Y    G    L    Q
351   GGACTGGAAG CCAGTCCTTA CGATAAACTC CATAATTTAT GGCCTGCAGT
      CCTGACCTTC GGTCAGGAAT GCTATTTGAG GTATTAAATA CCGGACGTCA
--------------------------------------------------------------------------------
+1    Y    L    F    L    E    P    N    P    E    D    P    L    N    K    E    A    A
401   ATCTCTTCTT GGAGCCCAAC CCCGAGGACC CACTGAACAA GGAGGCCGCA
      TAGAGAAGAA CCTCGGGTTG GGGCTCCTGG GTGACTTGTT CCTCCGGCGT
--------------------------------------------------------------------------------
+1    E    V    L    Q    N    N    R    R    L    F    E    Q    N    V    Q    R    S
451   GAGGTCCTGC AGAACAACCG GCGGCTGTTT GAGCAGAACG TGCAGCGCTC
      CTCCAGGACG TCTTGTTGGC CGCCGACAAA CTCGTCTTGC ACGTCGCGAG
--------------------------------------------------------------------------------
+1    M    R    G    G    Y    I    G    S    T    Y    F    E    R    C    L    K
501   CATGCGGGGT GGCTACATCG GCTCCACCTA CTTTGAGCGC TGCCTGAAAT
      GTACGCCCCA CCGATGTAGC CGAGGTGGAT GAAACTCGCG ACGGACTTTA
--------------------------------------------------------------------------------
+1    *
551   AG
      TC
--------------------------------------------------------------------------------
```

FIG. 2

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```
                20         40         60         80
yUbc12: MLKIRQIQK.KKQKENENSS.SIQPNLSAARIRLKRDLDSIDLPPIVTLNVITSPDSADRSQSPKLEVIVRPDEGMYNYGSINFN:83
NCE1  : MIKLFSIKQQKKEESAGGIKGSSKKASAAQIRIQKDINEINLPKTCDISFSD.PD.D...LLNFKLVICPDEGFYKSGKFVES  :79

                100        120        140        160
yUbc12: LDFNEVYPIEPPKVVQLKKIFHPNIDLKGNVCLNILREDWSPAIDLQSIITGLLFLFLEPNPNDPLNKDAAKIICEGEKEFAEAV:168
NCE1  : FKVGQGPHDPPKVKCETMVYHPNIDIFGNVCLNILREDWKPVITNSIIYGLQYLFLEPNPEDPLNKEAAEVLQNNRRIFEQNV  :164

                180
yUbc12: RLTMSGGSIEHVKYDNIVSP:188
NCE1  : QRSMRGGMIGSTYFERCIK.:183
```

FIG. 3

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FIG. 4

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```
+1     M   L   T   L   A   S   K   L   K   R   D   D   G   L   K   G   S
1      ATGCTAACGC TAGCAAGTAA ACTGAAGCGT GACGATGGTC TCAAAGGGTC
       TACGATTGCG ATCGTTCATT TGACTTCGCA CTGCTACCAG AGTTTCCCAG
----------------------------------------------------------------------
+1     R   T   A   A   T   A   S   D   S   T   R   R   V   S   V   R
51     CCGGACGGCA GCCACAGCGT CCGACTCGAC TCGGAGGGTT TCTGTGAGAG
       GGCCTGCCGT CGGTGTCGCA GGCTGAGCTG AGCCTCCCAA AGACACTCTC
----------------------------------------------------------------------
+1     D   K   L   L   V   K   E   V   A   E   L   E   A   N   L   P   C
101    ACAAATTGCT TGTTAAAGAG GTTGCAGAAC TTGAAGCTAA TTTACCTTGT
       TGTTTAACGA ACAATTTCTC CAACGTCTTG AACTTCGATT AAATGGAACA
----------------------------------------------------------------------
+1     T   C   K   V   H   F   P   D   P   N   K   L   B   C   F   Q   L
                                                 HindIII
                                                 ~~~~~~
151    ACATGTAAAG TGCATTTTCC TGATCCAAAC AAGCTTCATT GTTTTCAGCT
       TGTACATTTC ACGTAAAAGG ACTAGGTTTG TTCGAAGTAA CAAAAGTCGA
----------------------------------------------------------------------
+1     T   V   T   P   D   E   G   Y   Y   Q   G   G   K   F   Q   F
201    AACAGTAACC CCAGATGAGG GTTACTACCA GGGTGGAAAA TTTCAGTTTG
       TTGTCATTGG GGTCTACTCC CAATGATGGT CCCACCTTTT AAAGTCAAAC
----------------------------------------------------------------------
+1     E   T   E   V   P   D   A   Y   N   M   V   P   P   K   V   K   C
251    AAACTGAAGT TCCCGATGCG TACAACATGG TGCCTCCCAA AGTGAAATGC
       TTTGACTTCA AGGGCTACGC ATGTTGTACC ACGGAGGGTT TCACTTTACG
----------------------------------------------------------------------
+1     L   T   K   I   W   H   P   N   I   T   E   T   G   E   I   [C]   L
301    CTGACCAAGA TCTGGCACCC CAACATCACA GAGACAGGGG AAATATGTCT
       GACTGGTTCT AGACCGTGGG GTTGTAGTGT CTCTGTCCCC TTTATACAGA
----------------------------------------------------------------------
+1     S   L   L   R   E   H   S   I   D   G   T   G   W   A   P   T
351    GAGTTTATTG AGAGAACATT CAATTGATGG CACTGGCTGG GCTCCCACAA
       CTCAAATAAC TCTCTTGTAA GTTAACTACC GTGACCGACC CGAGGGTGTT
----------------------------------------------------------------------
+1     R   T   L   K   D   V   V   W   G   L   N   S   L   F   T   D   L
401    GAACATTAAA GGATGTCGTT TGGGGATTAA ACTCTTTGTT TACTGATCTT
       CTTGTAATTT CCTACAGCAA ACCCCTAATT TGAGAAACAA ATGACTAGAA
----------------------------------------------------------------------
+1     L   N   F   D   D   P   L   N   I   E   A   A   E   H   H   L   R
                                                 PstI
                                                 ~~~~~~
451    TTGAATTTTG ATGATCCACT GAATATTGAA GCTGCAGAAC ATCATTTGCG
       AACTTAAAAC TACTAGGTGA CTTATAACTT CGACGTCTTG TAGTAAACGC
----------------------------------------------------------------------
+1     D   K   E   D   F   R   N   K   V   D   D   Y   I   K   R   Y
501    GGACAAGGAG GACTTCCGGA ATAAAGTGGA TGACTACATC AAACGTTATG
       CCTGTTCCTC CTGAAGGCCT TATTTCACCT ACTGATGTAG TTTGCAATAC
----------------------------------------------------------------------
+1     A   R   *
551    CCAGATGA
       GGTCTACT
----------------------------------------------------------------------
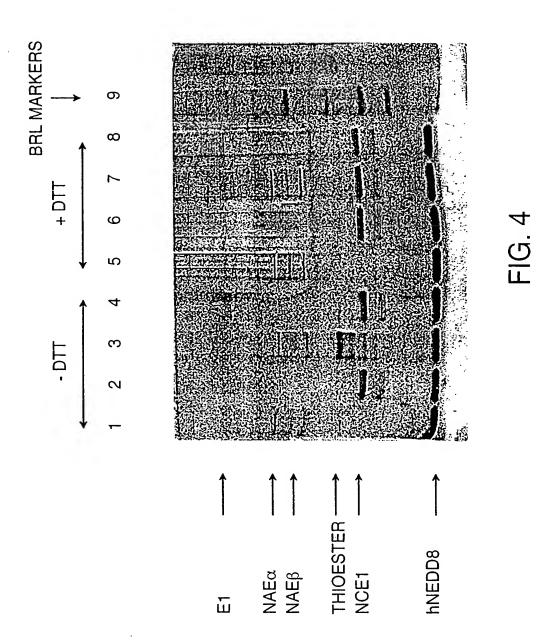```

FIG. 5

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FIG. 6

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FIG. 7A

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```
              *        180                  *        200            *          220            *          240          *
NCE1    :IIYGLQYDFLEP....NPEDPLNKEAAEVLQNNRRL.FEQNVQRSMRGGYIGSTYFERCLK............................:183
NCE2    :VVWGLNSDFRDLL....NFDDPLNIEAAEHHLRDKED.FRNKVDDYIKRYAR...............................:185
UBC1    :VLLSLQADLAAA.....EPDDPQDAVVANQYKQNPEM.FKQTARLWAHVYAGAPVSSPEYTKKIENLCAMGFDRNAVIVA:182
UBC2b   :ILTSIQSDLDEP....NPNSPANSQAAQLYQENKRE.YEKRVSAIVIQSWND.S.....................:152
UBC2a   :ILTSIQSDLDEP....NPNSPANSQAAQLYQENKRE.YEKRVSAIVIQSWRDC.....................:152
Cdc34a  :ILLSVISDLNEP....NTFSPANVDASVMYRKWKESKGKDREYTDIIRKQVLGTKVDAERDGVKVPTTLAEYCVKTKAPA:198
UB5B    :VLLSICSDLCDP....NPDDPLVPEIARIYKTDREK.YNRIAREWTQKYAM..............:147
UB5C    :VLLSICSDLCDP....NPDDPLVPEIARIYKTDRDK.YNRISREWTQKYAM..............:147
UB5A    :VLLSICSDLTDC....NPDDPLVPDIAQIYKSDKEK.YNRHAREWTQKYAM..............:147
UbcH6   :VLLSICSDLTDC....NPADPLVGSIATQYMTNRAE.HDRMARQWTKRYAT..............:193
UbcH7   :VIQSLIADVNDP....QPEHPLRADLAEEYSKDRKK.FCKNAEEFTKKYGEKRPVD.........:154
UbcH8   :VLFALNVDVNRP....NTREPLRMDIADLLTQNPEL.FRKNAEEFTLRFGVDRP.S........:152
UBE2G   :IMISVISMLADP....NGDSPANVDAAKEWREDRNGEFKRKVARCVRKSQETAFE.........:170
UBCH(8) :IFESFLPQLLAYP....NPIDPLNGDAAAMYLHRPEE.YKQKIKEYIQKYATEEALKEQEEGTGDSSSESSMSDFSEDEA:178
UBC9    :ILLGIQEDLNEP....NIQDPAQAEAYTIYCQNRVE.YEKRVRAQAKKFAPS............:158
UBCH10  :ILLSIQSDLGEP....NIDSPLNTHAAELWKNPTAF.K.KYLQETYSKQVTSQEP..........:180
UBC13   :VLLSIQADLSAP....NPDDPLANDVAEQWKTNEAQ.AIETARAWTRLYAMNNI...........:152


              *        260            *        280            *        300            *        320            *
NCE1    :.........................................................................
NCE2    :LSSKSWDVETATELLISN.......................................................:200
UBC1    :.........................................................................
UBC2b   :.........................................................................
UBC2a   :.........................................................................
Cdc34a  :PDEGSDLFYDDYYEDGEVEEEADSCFGDEDDSGTEES....................................:236
UB5B    :.........................................................................
UB5C    :.........................................................................
UB5A    :.........................................................................
UbcH6   :.........................................................................
UbcH7   :.........................................................................
UbcH8   :.........................................................................
UBE2G   :.........................................................................
UBCH(8) :QDMEL....................................................................:183
UBC9    :.........................................................................
UBCH10  :.........................................................................
UBC13   :.........................................................................
```

FIG. 7B

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FIG. 7C

LANE 1: NO NCE
LANE 2: + NCE1
LANE 3: + NCE2; ARROW INDICATES Nedd8 THIOESTER OF NCE2
LANE 4: SAME AS LANE 2 BUT + DTT
LANE 5: SAME AS LANE 2 BUT + DTT

FIG. 8